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# Letter to the Editor

## A Closer Look at the Canonical 'Raft Mixture' in Model Membrane Studies

Recently, Dietrich et al. (2001) reported lateral separation of liquid phases in model bilayer membranes of two lipid mixtures. One of their mixtures, 1:1:1 DOPC/brain sphingomyelin (BSM)/Cholesterol, has since become a canonical 'raft mixture' on which other groups have based their research. A recent example from this journal is a paper by Gandhavadi et al. (2002). One result presented by the authors is that lateral separation into two liquid phases could not be observed in bilayers of this mixture using x-ray diffraction techniques. The goal of this letter is to provide a possible explanation for this discrepancy between the two papers.

In recent work from our laboratory, we have shown that lateral separation into liquid phases in bilayer membranes is not limited to the small set of previously published model mixtures, but in fact can be attained over a large composition and temperature range (Veatch and Keller, 2002). In experiments using ternary lipid mixtures of equimolar saturated and unsaturated phosphatidylcholines mixed with cholesterol, coexistence of two liquid phases is observed in giant unilamillar vesicles (GUVs) with cholesterol compositions between 10–50 mol%. Domains are seen by fluorescence microscopy using 0.8 mol% Texas Red di(16:0)PE as a dye which partitions into the cholesterol-poor phase (Veatch and Keller, 2002). At high temperatures all lipids are in one uniform liquid phase so that no domains are observed. As temperature decreases through the miscibility transition, lipid domains form spontaneously.

A typical phase diagram of the 1:1 DOPC/BSM + cholesterol mixture is shown in Fig. 1 *a*. At low cholesterol concentrations (~10–25 mol%), miscibility transition temperatures are highest and do not vary greatly with cholesterol composition. At higher cholesterol, (~30–35 mol% cholesterol) the miscibility transition temperature decreases sharply until a composition is reached (>35–40 mol%) at which domains are no longer observed in vesicles over accessible temperatures. Phase diagrams for similar ternary lipid mixtures (including sphingomyelin) follow this general trend with minor differences (Veatch and Keller, 2002).

Considering the general phase behavior of these types of mixtures, there are three major reasons why the canonical 1:1:1 DOPC/BSM/Cholesterol 'raft mixture' can be problematic. First, this mixture is poised close to the high cholesterol edge of the miscibility transition. Since this edge is steep, slight changes in lipid composition at a given laboratory temperature will change the phase behavior. The second problem arises because different experimental methods produce bilayers with slightly different distributions of compositions. Again, since the high cholesterol edge of the miscibility transition is steep, this slight variation in composition will result in a large variation in observed phase behavior between experiments. Third, since miscibility transition temperatures are generally lower at higher cholesterol composition, it is likely that the transition in the 1:1:1 mixture will occur near or below room temperature. Hence, coexisting liquid domains will not necessarily be observed under normal experimental conditions. (In Dietrich et al. (2001), 1% GM1 was included in their 1:1:1 vesicles.) Combining all of these trends, it is clear that small changes in experimental conditions lead to large changes in the observed phase behavior for this particular mixture.

To illustrate this point, and to support the use of Fig. 1 *a*, we provide fluorescence micrographs of 1:1:1 DOPC/BSM/cholesterol GUVs at 25°C in Fig. 1 *b* and at 20°C in Fig. 1 *c*. The same field of view is shown in both frames. In both cases, lateral separation of domains is observed in some but not all vesicles. Vesicles with domains are shown with arrows. Coexisting domains on the surface of a vesicle are clearly distinguished from small vesicles in the interior. First, when the focus is adjusted to be midway through a giant vesicle, surface domains are no longer seen although interior vesicles are. Second, domains on the surface disappear as temperature is raised and interior vesicles remain. In Fig. 1 *b* at 25°C many vesicles are in one uniform phase (above the transition) whereas in Fig. 1 *c* at 20°C the majority exhibit coexisting liquid phases on their surfaces (below the transition). The range in transition temperatures is due to the slight variation in composition between vesicles. For our method of making GUVs (Angelova et al., 1992), we estimate a variation of ±2 mol% cholesterol between individual vesicles. For this reason, we always examine large populations of vesicles.

The observations above confirm that, indeed, at room temperature this 1:1:1 DOPC/BSM/cholesterol mixture is close to the miscibility transition temperature and that small changes in vesicle composition have a large effect on the observed phase behavior. In contrast, all vesicles made from 1:1 DOPC/BSM with 20% cholesterol exhibit two liquid phases at 25°C (Fig. 1 *d*) indicating that this mixture may be a better choice for most experiments. In addition, the cholesterol-poor phase makes up the majority of these membranes. This may more closely resemble biological membranes in which cholesterol rich 'rafts' are islands in a background of liquid disordered phase.

These results indicate that it is important to consider the lipid composition and corresponding miscibility transition temperature of 'raft forming mixtures' used in experiments. This lesson is especially important at this time when many

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Address reprint requests to Sarah L. Keller, Dept. of Chemistry, Box 351700, University of Washington, Seattle, WA 98195-1700. Tel.: 206-543-9613.
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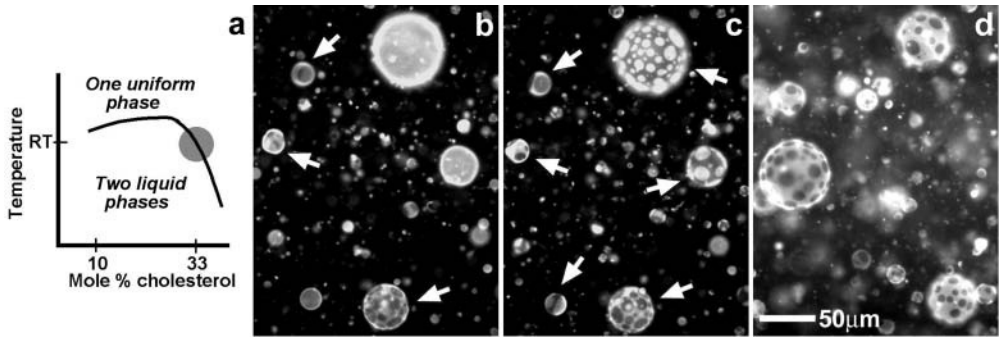


FIGURE 1 (*a*) Simplified sketch of anticipated miscibility phase diagram for mixtures of 1:1 DOPC/BSM with varying cholesterol composition. The location of the popular mixture of 1:1:1 DOPC/BSM/cholesterol at room temperature (*RT*) is denoted by a gray circle. (*b, c*) GUVs of 1:1:1 DOPC/BSM/cholesterol at 25°C (*b*) and 20°C (*c*). The same field of view is shown in both frames. In both cases, laterally separated liquid domains are observed in some but not all vesicles. Vesicles with domains are shown with arrows. At 20°C, more vesicles exhibit coexisting domains than at 25°C. (*d*) All vesicles made with 1:1 DOPC/BSM and 20% cholesterol exhibit phase separation at 25°C. All experiments use the fluorescent dye Texas Red DPPE which preferentially partitions into the cholesterol-poor phase.

groups are working to characterize and better understand the mechanism and physical nature of these laterally phase separated systems.

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Sarah L. Veatch and Sarah L. Keller

Departments of Chemistry and Physics, University of Washington, Seattle WA 98195-1700